Environmental Health Perspectives
Vol. 22, pp. 131–132, 1978

Use of Cultured Human Cells in Short-Term Tests for Chemical Carcinogens

by H. F. Stich,* R. H. C. San,* P. Lam,* and J. Koropatnick*

Following the recent explosion in the number of designs for short-term assays for mutagens and carcinogens it appears worthwhile to take stock of the current situation and speculate about future developments. Over 400 strong, weak, and noncarcinogenic compounds have been examined by one or the other of the microbial tests, Drosophila assay, or the DNA fragmentation/DNA repair system of cultured mammalian cells. Between 80 and 90% of examined carcinogens give a positive response if checked with one test. If the results of several tests are pooled, only a very few carcinogens escape detection. In spite of these highly promising results, there is a reluctance on the part of regulatory agencies to accept wholeheartedly the rapid, inexpensive, and reproducible short-term bioassays. Why? The phylogenetic relationship between bacteria and man appears for some too remote to permit an extrapolation from microbial tests to human populations. Others doubt the validity of using point mutations as an endpoint, claiming that mutations may not play a role in neoplastic transformation and thus cannot be used as a relevant endpoint.

One answer to these heretics could be to rank the various endpoints including, of course, carcinogenicity and to calculate a coefficient of rank correlation. Another answer would be to use several well chosen, short-term assays which complement each other. The Salmonella mutagenicity test in combination with activation systems (*1*) and the recombination assay of Kada (*2*) can be applied to mutagens/carcinogens found in the environment, in complex mixtures, or in body fluids of man. DNA fragmentation/DNA repair assays on cultured human cells (*3*) can be used to simulate conditions prevailing in man, and should, therefore, produce data of high relevance to human populations (*4*). DNA fragmentation in various organs of mice or rats fed the carcinogenic compounds (*5*) appears to incorporate the convenience of *in vitro* assays with the completeness of the metabolic activation/deactivation system of the whole animal.

In this short report we would like to point to several advantageous features of using cultured human cells as indicators and DNA fragmentation/DNA repair as endpoints. The outstanding advantage of this test system is its adaptability to situations which prevail in man, thus permitting an easy transfer of *in vitro* results to *in vivo* conditions. A few examples should illustrate this point.

Carcinogenic nitrosation products can be readily detected when nitrosation is carried out in simulated gastric juice (*6*). Genetically controlled susceptibility can be uncovered by estimating the level of repair synthesis following various chemical carcinogens (*7*) or the reactivation of ultraviolet-irradiated DNA viruses (*8, 9*). If these techniques are combined with cell survival studies, variations in sensitivity within human population groups could be detected.

The susceptibility of human cells to chemical carcinogens can be enhanced by interfering with their DNA repair capacity. The effect of ascorbic acid on DNA repair synthesis and cell survival following exposure to the potent carcinogen 4NQO exemplifies this pattern (*10*).

Most screening procedures are based on response to single doses. However, the average man in an industrial society is repeatedly exposed to various carcinogens. A preliminary study on the effect of two consecutive doses revealed a "refractive period" following the application of a carcinogenic

*Cancer Research Centre and Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

agent (UV, 4NQO or MNNG). During this period, the cell cannot respond to a second dose with a normal level of DNA repair synthesis. In this stage the cell temporarily acquires an elevated sensitivity to the chromosome damaging action and lethal effect of chemical mutagens/carcinogens (*11*).

The interaction of chemical and physical agents is another area which has received less attention than it merits, e.g., the combined use of psoralen and long ultraviolet-radiation in the treatment of psoriasis. DNA fragmentation and DNA repair of epidermal cells can be employed to assess the potential hazard of this therapy to the genome of somatic cells. To what extent photosensitizing chemicals (*12*) enter man and sensitize him to the action of radiation is difficult to assess at present.

The use of human cells also offers the opportunity to study the effect of human viruses on the response of cells toward chemical carcinogens. All the variations found in nature can be easily simulated in the tissue culture systems. Cells with an infectious virus replicative cycle or with abortive infection, cells with a latent virus, and cells with an integrated viral genome can be exposed to chemical carcinogens and their response compared to that of noninfected cells. A profound understanding of the virus–chemical carcinogen interactions should greatly improve the predictive value of short-term assays.

Finally, the complex interplay between chemical carcinogens, viruses and genetic composition of the target cells must be unravelled to understand the factors which enhance or reduce the risk of neoplastic transformation (*13*).

In spite of the many advantages offered by the above-mentioned bioassays, there are several unresolved issues that need attention. For the sake of simplicity we have used fibroblasts obtained from small skin biopsies of normal and genetically-afflicted persons of high cancer risk. Thus all our studies are done on fibroblasts instead of the epidermal cells from which most human tumors arise. Criticism can also be directed toward the exclusive use of cultured cells forced to grow on glass or plastic surfaces. The possibility of whether human organ pieces could be grown in immunodepressed mice which are then exposed to chemical carcinogens should be explored. This approach, if successful, would have the advantage of having human target tissue in its original form treated with chemical carcinogens.

In our opinion there is at present little to gain in the continuous introduction of new short term assays or new indicator subjects. We would rather favor the selection of a very few bioassays and proceed with their in depth analysis. Serious consideration should be given to: (1) bioassays which are the most rapid, economic and reproducible for the large-scale screening of carcinogens in man's environment and (2) bioassays which can simulate closest the conditions prevailing in man. The combination of both groups of bioassays should increase their predictive value in assessing carcinogenic hazards to human populations, and should find acceptance by regulatory agencies.

REFERENCES

1. McCann, J., et al. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Nat. Acad. Sci. (U. S.), 72: 5135 (1975).
2. Shirasu, Y., et al. Mutagenicity screening of pesticides in the microbial system. Mutat. Res. 40: 19 (1976).
3. San, R. H. C., and Stich, H. F. DNA repair synthesis of cultured human cells as a rapid bioassay for chemical carcinogens. Int. J. Cancer, 16: 284 (1975).
4. Stich, H. F., et al. DNA fragmentation and DNA repair as an *in vitro* and *in vivo* assay for chemical precarcinogens, carcinogens and carcinogenic nitrosation products. Paper presented at IARC/CEC Workshop on Rapid Screening *Tests to Predict Late Toxic Effects of Environmental* Chemicals, Brussels, June 1975.
5. Laishes, B. A., Koropatnick, D. J., and Stich, H. F. Organ-specific DNA damage induced in mice by the organotropic carcinogens 4-nitroquinoline-1-oxide and dimethylnitrosamine. Proc. Soc. Exptl. Biol. Med. 149: 978 (1975).
6. Lo, L. W., and Stich, H. F. DNA damage, DNA repair and chromosome aberrations of xeroderma pigmentosum cells and controls following exposure to nitrosation products of methylguanidine. Mutat. Res. 30: 397 (1975).
7. Stich, H. F., San, R. H. C., and Kawazoe, Y. Increased sensitivity of xeroderma pigmentosum cells to some chemical carcinogens and mutagens. Mutat. Res. 17: 127 (1973).
8. Day, R. S. Xeroderma pigmentosum variants have decreased repair of UV-damaged DNA. Nature 253: 748 (1975).
9. Stich, H. F., Stich, W., and Lam, P. Susceptibility of xeroderma pigmentosum cells to chromosome breakage by adenovirus type 12. Nature, 250: 599 (1974).
10. Stich, H. F., et al. Mutagenic action of ascorbic acid. Nature, in press.
11. Warren, P. M., and Stich, H. F. Reduced DNA repair capacity and increased cytotoxicity following split doses of the mutagen 4-nitroquinoline-1-oxide in cultured human cells. Mutat. Res. 28: 285 (1975).
12. Stich, H. F., et al. The search for relevant short term bioassays for chemical carcinogens: the tribulation of a modern Sisyphus. Can. J. Genet. and Cytol. 17: 471 (1975).
13. Stich, H. F., Hammerberg, O., and Casto, B. The combined effect of chemical mutagen and virus on DNA repair, chromosome aberrations, and neoplastic transformation. Can. J. Genet. Cytol. 14: 911 (1972).